MICELLULA DNA EMULSION & PURIFICATION KIT

roboklon

EURx

QUICK MANUAL

KIT VERSION 1.0, JULY 2010. SEE ALSO DETAILED VERSION OF THIS PROTOCOL.

A EMULSION REACTION SET-UP

1. Emulsion - Setting up the Oil Phase

→ Prepare 300 µl Oil Surfactant Mixture per reaction (50 µl water phase). Mix
220 µl Emulsion Component 1 (73 % [v/v])
20 µl Emulsion Component 2 (7 % [v/v])
60 µl Emulsion Component 3 (20 % [v/v])

→ Mix thoroughly by vortexing.

→ Keep on wet ice until further usage.

2. Emulsion - Setting up the Water Phase

→ Prepare 50 µl Water Phase per Emulsion reaction (e.g. ePCR).
Mix template DNA and enzyme without BSA according to application requirements.

→ Remove aliquot for "open" (unemulsified) reaction control (optional).

→ Add BSA. Required amount: _____

3. Create Emulsion Reactions

→ Mix 300 µl Oil Surfactant Mixture (precooled, 4°C) and 50 µl Water Phase.

→ Vortex thoroughly for 5 minutes at 4°C on a vortexer with fixation aperture.

→ Dispense emulsion to three thin-walled reaction tubes ("triplicates") and run reaction.

B BINDING STEP – DNA PURIFICATION

4. Emulsion Breaking and DNA Binding Step

→ Apply 40 µl Activation Buffer DX to spin-column membrane,
keep column at room temperature until usage, do not spin.

→ Preheat Elution Buffer to 80°C (see section D, Elution).

→ Pool triplicates of each sample into a 2 ml plastic reaction tube.

→ Add 1 ml 2-butanol (or butanol) and break emulsion by vortexing.

→ Add 400 µl of orange-colored Orange-DX buffer (max. 250 µl water phase / 25 µg DNA).

→ Mix buffer completely with sample.

→ Centrifuge for 2 min at 11 000 x g (approx.12 000 rpm).

→ Remove organic phase (leave a small remain, do not remove interphase).

→ Transfer water phase, interphase and remains of organic phase
into a spin-column / receiver tube assembly.

→ Centrifuge for 1 min at 11 000 x g (approx. 12 000 rpm).

C WASHING STEPS – DNA PURIFICATION

5. First Washing Step

→ Discard flow-through and place back spin-column.

→ Add 500 µl of Wash-DX1 buffer to spin-column.

→ Centrifuge for 1 min at 11 000 x g (approx.12 000 rpm).

6. Second Washing Step

→ Discard flow-through and place back spin-column.

→ Add 650 µl of Wash-DX2 buffer to spin-column.

→ Centrifuge for 1 min at 11 000 x g (approx.12 000 rpm).

7. Removal of Wash Buffer Traces

→ Discard flow-through and place back spin-column.

→ Centrifuge for 2 min at 11 000 x g (approx.12 000 rpm)
to remove any remaining traces of Wash-DX2 buffer.

D ELUTION STEP – DNA PURIFICATION

8. DNA Elution

→ Place spin-column in new collection tube (1.5 - 2 ml).

→ Add 50-150 µl Elution-DX buffer (optional: heated to 80°C).

→ Incubate for 2 min at room temperature.

→ Centrifuge for 1 min at 11 000 x g (approx. 12 000 rpm).

→ Discard spin-column, cap the collection tube.
DNA is ready for analysis / manipulation or scale-up emulsion reaction.

→ Store DNA at -20°C (recommended) or at 4°C (short term only).

THIS PROTOCOL IS AVAILABLE ONLINE

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